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BIOSYSTEMATICS OF AEDES (NEOMELANICONION)

Annual Report

Thomas J. Zavortink

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19. Abstract

The objective of the "Biosystematics of Aedes (Neomelaniconion)" project is to produce a modern taxonomic monograph of the aedine subgenus Neomelaniconion. Comparative morphological taxonomic procedures will be emphasized. Characteristics from both sexes and all stages of the life cycle will be studied.

During the fourth contract year, the primary types of 10 nominal species of Neomelaniconion from the British Museum (Natural History) and the Musee Royal de L'Afrique Centrale were studied. No field surveys were made. Cooperators provided live eggs of five species from Kenya and South Africa. Some adults reared from these eggs were used to develop a force-mating technique, and this technique was used to maintain strains of four Savanna Group species in the laboratory and to produce hybrids between these species. Approximately 1,350 adult mosquitoes, 800 slides of immature mosquitoes, and 39 slides of male genitalia were prepared for morphological study. Approximately 950 adult Neomelaniconion were frozen for electrophoretic study. A complete set of preliminary drawings of the larva, pupa, and male genitalia of four species of Neomelaniconion were completed. All preliminary drawings of larvae and pupae made for the project to date (20 species) were checked and corrected for the modal number of branches in each seta. All available specimens of Neomelaniconion were re-examined and some earlier identifications changed. Provisional keys to the females and male genitalia of most Ethiopian species and a table of the distributions of these species were prepared. The phylogenetic relationships of most species of Neomelaniconion were explored by cladistic analysis of adult morphological characteristics with the PAUP computer program. An electrophoretic study of soluble cellular enzymes of 17 populations of six Savanna Group species was performed, and the resulting genetic information analyzed by the BIOSYS-1 and FREQPARS computer programs. The cluster diagram and cladogram produced by these two analyses were incongruent with each other and with the cladogram produced from morphological characteristics of adults. The validity of the five currently-recognized species included in the BIOSYS-1 analysis was confirmed by the electrophoretic data. The F_1 hybrids between the Savanna Group species produced in the laboratory are at least partly fertile, so that F_2 and backcross progeny can be obtained.

Contents

Statement of the Problem

The goal of the project "Biosystematics of Aedes (Neomelaniconion)" is to produce a modern taxonomic monograph of this subgenus of mosquitoes. Neomelaniconion, which is primarily Ethiopian in distribution, has not been studied carefully, and so its species are poorly known. The absence of basic information on the number of species and on how to distinguish them severely hampers the acquisition and reporting of biological information about these mosquitoes. The result is that the distribution, bionomics, and disease vector potential of the different species remain unknown or uncertain.

Species of Neomelaniconion are believed to be involved in both the inter-epizootic maintenance and transmission of Rift Valley fever virus. A complete understanding of the natural history of this virus is not possible without better knowledge of these mosquitoes.

Background

As it is presently understood, the subgenus Neomelaniconion includes 28 nominal species, 24 of which are considered to be valid taxonomic species or subspecies (1-3). All except one of the currently recognized species are restricted to the Ethiopian Region. The exception is Aedes lineatopennis (Ludlow), which is widespread in the Oriental and Australian regions.

The existing taxonomy of the subgenus Neomelaniconion dates back to Edwards's treatment of the group under its former name, Banksinella Theobald, in his catalog of the family Culicidae (4) and in his volume on Mosquitoes of the Ethiopian Region (5). Edwards's studies were based almost entirely upon adult mosquitoes, and characteristics of the immature stages were not considered. In the many decades since Edwards's brief taxonomic treatments of Neomelaniconion, there has been no comprehensive study of the group. Several additional species have been described (3, 6-10), immatures of a few species have been partially described or illustrated (7, 9, 11-17), one nominal species has been transferred to the subgenus (18), and two nominal species have been removed (19).

In the absence of a comprehensive study of Neomelaniconion, the subgenus remains poorly and inadequately known. The immature stages, in particular, have been neglected. They have never been used to help define the species of the group or to help place these species into a natural classification. In fact, to this day the immatures

of nearly half the species of _Neomelaniconion_ are unknown, and for those species in which they are known, they have been described and illustrated very superficially. The complete larval and pupal chaetotaxy has not been studied for a single species. Available keys to adults (5, 9, 15) and larvae (11, 15) of _Neomelaniconion_ are inadequate because they treat only a portion of the species now known or treat only the species of a restricted region.

Numerous arboviruses have been isolated from species of _Neomelaniconion_ (20). The virus that causes Rift Valley fever, an important disease of domestic animals and humans in Africa and a potential international disease problem (21), is the most important of these. This virus has been isolated from field populations of three or more species of _Neomelaniconion_: _circumluteolus_ (Theobald) in South Africa (22) and Uganda (23); _lineatopennis_ in Kenya (24), South Africa (25), and Zimbabwe (26); _palpalis_ (Newstead) in Central African Republic (27); and possibly _luteolateralis_ (Theobald) in South Africa (28). Laboratory experiments have shown that the virus can be transmitted horizontally by yet another species of _Neomelaniconion_, _unidentatus_ McIntosh (29). Studies of Rift Valley fever in Kenya have provided evidence that _lineatopennis_ is a reservoir for the virus between epizootics, transmitting it transovarially from generation to generation (30). The identity of the _Neomelaniconion_ species reported as _lineatopennis_ in all of these studies is in doubt; in Kenya the species is probably the recently described _mcintoshi_ Huang (3), but in South Africa it may be an undescribed sibling species in this complex. The fact that Rift Valley fever virus has been isolated from several species of _Neomelaniconion_ and is known to be transmitted horizontally or vertically by some of these mosquitoes underscores the importance of obtaining basic information on the systematics and biology of species of _Neomelaniconion_, for such information is critical to a complete understanding of the natural history of Rift Valley fever virus.

Approach to the Problem

A modern systematic study of _Neomelaniconion_, utilizing morphological characteristics from both sexes and all stages in the life cycle, will be undertaken in order to determine the number of species in the subgenus, the most reliable means of distinguishing these species from each other, the existence and nature of intraspecific variation, the geographic distribution of the species, and the evolutionary relationships of the species. The results of this study will be published in a monograph that will include: taxonomic descriptions of species and groups of species;

identification keys for all stages in the life cycle; detailed drawings of the larva, pupa, and male genitalia of each species and of the adult morphology for selected species; photographs of eggs; information on type specimens; synonymies; discussions of diagnostic characters, variation, and relationships; summaries of bionomics and medical importance; data on geographical distribution of the species, including lists of specimens examined and maps; and a bibliography.

Although the historically important specimens of Neomelaniconion currently held in museums will be examined, the bulk of the specimens studied will be collected specifically for the project. The collection, rearing, and preservation of material and the recording of field data will follow the procedures developed for the "Mosquitos of Middle America" project (31). Emphasis will be placed on collecting adult females from which eggs for progeny rearings can be obtained and on collecting the immature stages so they can be reared individually. Both progeny rearings and individual rearings associate the stages of a species, and progeny rearings associate the sexes unequivocally. Specimens collected in the field or borrowed from museums will be prepared for study using standard laboratory procedures for mosquitoes, in general following the methods of Belkin (19). Classical, comparative morphological taxonomic procedures will be emphasized, as outlined for mosquito systematics by Belkin (19) and Zavortink (32). The form of presentation and terminology used in the final monograph will follow Belkin (19) and Zavortink (33-35) in large part.

Results and Discussion

Accomplishments related to the goal of producing a monograph of the subgenus Neomelaniconion that were completed during the fourth contract year of the project "Biosystematics of Aedes (Neomelaniconion)" are described below.

STAFF

The following staff were supported by the contract during the fourth year:

- Thomas J. Zavortink, Principal Investigator (50% time)
- Sandra S. Shanks, Taxonomic Research Specialist (100% time May through November 1989; 20% time February through April 1990)
- Mary Ann Tenorio, Taxonomic Research Specialist (80% time December 1989 through April 1990); Scientific Illustrator (Piecework)

COOPERATORS

The following individuals contributed to the "Biosystematics of *Aedes* (*Neomelaniconion*)" project during the fourth contract year:

Anton Cornel, South African Institute for Medical Research, Johannesburg, South Africa, brought live eggs of *Neomelaniconion* from South Africa to San Francisco.

George B. Craig, Jr., University of Notre Dame, Notre Dame, Indiana, allowed the Principal Investigator to utilize his staff, equipment, and supplies at the Vector Biology Laboratory, University of Notre Dame, in order to obtain electrophoretic data for several species of *Neomelaniconion*.

Peter Jupp, National Institute for Virology, Johannesburg, South Africa, sent live eggs of *Neomelaniconion* from South Africa.

Kenneth J. Linthicum, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, sent live eggs of *Neomelaniconion* from Kenya.

Leonard E. Munstermann, University of Notre Dame, Notre Dame, Indiana, taught the Principal Investigator electrophoretic techniques and supervised the gathering of electrophoretic data for several species of *Neomelaniconion*.

ACQUISITION OF SPECIMENS

Loans from Museums. - The primary types of 10 nominal species of *Neomelaniconion* were borrowed from the British Museum (Natural History) and the Musee Royal de L'Afrique Centrale, Tervuren, Belgium.

Collecting and Rearing. - No field surveys were conducted by the Project's staff during the contract year. However, live eggs of four species of *Neomelaniconion* - *aurovenatus* Worth, *circumluteolus*, *luteolateralis*, *mcintoshi* - were received through cooperators in South Africa, and live eggs of three species - *circumluteolus*, *mcintoshi*, *unidentatus* - were received from Kenya. Much valuable material has been reared from these eggs; some has been prepared for morphological study, some has been frozen for electrophoretic study, and some has been used to develop force-mating techniques.

The last clutches of eggs obtained in Central African Republic in 1988 were flooded early in the fourth contract year and the larvae that hatched were reared.

No additional experimentation with the rearing techniques developed during the third contract year has been done. However, the concentration of nutrient broth used to

stimulate egg hatching through promotion of bacterial growth reported in the Third Annual Report is incorrect. The concentration is 0.01%, not 0.1%.

The Principal Investigator learned force-mating techniques for mosquitoes at the Vector Biology Laboratory, University of Notre Dame, during periods of study there in January 1988 and July 1989. Experimentation with several species of Neomelaniconion in the Savanna Group has shown that these mosquitoes can be force-mated successfully. Four species - circumluteolus, luteolateralis, mcintoshi, and unidentatus - have been maintained through two or three generations in the laboratory. Of these, circumluteolus is the best candidate for a laboratory animal; adults can be force-mated easily, females feed readily on human blood, lay eggs readily on crumpled laboratory tissue moistened with distilled water, and are long-lived. At 25°C, females can complete a gonotrophic cycle every three days and can lay as many as 12 clutches of eggs. The three other species are more difficult to maintain because they are harder to mate (mcintoshi, unidentatus), less inclined to feed on human blood (unidentatus), require some unknown ovipositional stimulant (luteolateralis, unidentatus), or live for a shorter period of time (unidentatus).

The force-mating technique used for Neomelaniconion is as follows: 1) adults are held for two to seven days after eclosion and are provided with a source of carbohydrate (sucrose solution or moistened raisin) during this time; females may be offered a blood meal, but relatively few will feed; 2) males are anesthetized with ether only as long as it takes to knock them down (about 30 seconds); 3) anesthetized males are impaled on minuten pins inserted into wooden applicator sticks, with the minuten entering the thorax of the mosquito in the lower left sternopleural area and exiting through the scutum; 4) the head and hind legs of the impaled males are removed with fine forceps; 5) decapitated males are held over moistened towels for several minutes while they recover from the anesthesia; 6) females whose abdomens are distended by feeding on carbohydrate or blood are anesthetized with ether for three or four minutes; 7) an anesthetized female is placed on its back on a large rubber eraser so that its genital segments extend over the edge of the eraser; 8) a decapitated male impaled on a minuten pin is held so that its ventral surface is up and is maneuvered into position so that the cerci of the female extend between the sidepieces of the genitalia of the male; if the male is one that displays a strong mating response, then it will clasp the female with movements of its claspers and sidepieces and insert its aedeagus into the genital atrium of the female. Copulation is brief, lasting only 10-15 seconds, and during this time the female is held so firmly by the male that it

can be picked up, suspended only by the genitalia of the male. Males that readily mate can be mated to more than one female. Males that do not mate should be tried several times over a period of several minutes before being discarded. Steps 3, 4, 7, and 8 of the force-mating technique are performed at 10 to 20 times magnification under a stereoscopic microscope.

Force-mating techniques have been used to obtain hybrids between several species in the Savanna Group. In some instances, F_2 hybrids and/or backcrosses of the F_1 hybrids to one or both parental species have been produced. To date, progeny have been reared from each of the following crosses (the female parent in each cross is listed first): *circumluteolus* x *luteolateralis*; *circumluteolus* x *unidentatus*; *mcintoshi* x *circumluteolus*; *mcintoshi* x *luteolateralis*; *unidentatus* x *circumluteolus*; *unidentatus* x *luteolateralis*; *unidentatus* x *mcintoshi*; (*mcintoshi* x *circumluteolus*) F_2; (*mcintoshi* x *luteolateralis*) F_2; (*unidentatus* x *mcintoshi*) F_2; *circumluteolus* x (*circumluteolus* x *luteolateralis*); (*circumluteolus* x *luteolateralis*) x *circumluteolus*; (*circumluteolus* x *luteolateralis*) x *luteolateralis*; *circumluteolus* x (*mcintoshi* x *circumluteolus*); (*mcintoshi* x *circumluteolus*) x *circumluteolus*; and (*mcintoshi* x *luteolateralis*) x *luteolateralis*. The following hybridizations have been made, but the eggs have not been flooded yet: *circumluteolus* x *mcintoshi*; (*circumluteolus* x *luteolateralis*) F_2.

Live or frozen material produced in the laboratory at the University of San Francisco has been provided to other investigators. Live eggs of *circumluteolus*, *mcintoshi*, and *unidentatus* were shipped to Kenneth Linthicum, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland. Frozen adults of the same three species were shipped to Linda Strausbaugh, University of Connecticut, Storrs, Connecticut, for use in development of histone DNA probes. Numerous backcross adults have been frozen and are available to other researchers for genetic analysis.

PREPARATION OF SPECIMENS FOR STUDY

All adult mosquitoes reared for morphological study during the fourth contract year have been mounted for study. Among these are specimens reared from eggs obtained in Central African Republic in 1988, specimens reared from eggs obtained in Kenya and South Africa by cooperators in 1989, and specimens of the various F_1, F_2, and backcross hybrids mentioned in the previous section. Most larval and pupal exuviae and whole larvae from these same collections have been slide-mounted. All of this material must still be

labeled with permanent, printed locality labels. A large backlog of material reared during the second and third contract years also remains to be labeled.

During the fourth contract year, approximately 1350 adult _Neomelaniconion_ were mounted on points; approximately 800 microscope slides of immature _Neomelaniconion_ (whole larvae and larval and pupal exuviae) were prepared; 39 microscope slides of male genitalia of _Neomelaniconion_ were prepared; and approximately 950 adult _Neomelaniconion_ were frozen for use in molecular systematic and genetic studies.

IDENTIFICATION

All species of _Neomelaniconion_ reared from eggs during the fourth contract year have been identified. These species and their geographic origins are:

Aedes (_Neomelaniconion_)
- _aurovenatus_ South Africa
- _circumluteolus_ Kenya, South Africa
- _luteolateralis_ South Africa
- _mcintoshi_ Kenya, South Africa
- _palpalis_ Central African Republic
- _taeniarostris_ (Theobald) Central African Republic
- _unidentatus_ Kenya

During the fourth contract year, some of the provisional identifications of _Neomelaniconion_ in the British Museum (Natural History) and United States National Museum collections reported in the First Annual Report were changed, and the names used by me for species in the _carteri_ complex in the Second and Third Annual Reports were changed. These changes are discussed in more detail in the Taxonomic Study section.

ILLUSTRATION

During the fourth contract year preliminary pencil drawings of the larva, pupa, and male genitalia of four species of _Neomelaniconion_ were completed.

All drawings of larvae and pupae made for the project have been checked by the illustrator and corrected for the modal number of branches in each seta compiled from a sample of specimens. These drawings await final checking by the Principal Investigator before inking.

No additional scanning electron micrographs of eggs of _Neomelaniconion_ were prepared.

A summary of all illustrations of _Neomelaniconion_ prepared

for the project by the end of the fourth contract year is: a scanning electron micrograph of the egg has been prepared for 18 species; a complete set of preliminary drawings of the larva, pupa, and male genitalia has been prepared for 20 species; and a preliminary drawing of the male genitalia has been prepared for two additional species.

TAXONOMIC STUDY

During the fourth contract year the primary types of 10 nominal species of Aedes (Neomelaniconion) were examined. These were: albicosta (Edwards), bequaerti Wolfs, carteri Edwards, chrysothorax (Theobald), circumluteolus, crassiforceps Edwards, luteolateralis, palpalis, pogonurus Edwards, and taeniarostris.

Study of the type of carteri has shown that the species of Neomelaniconion collected in Ivory Coast and reported as carteri in the Second and Third Annual Reports for the project is actually undescribed, and that the species from Central African Republic called undescribed species number 8 in the Third Annual Report is carteri. The undescribed species from Ivory Coast is now referred to as undescribed species number 10. Study of the extant syntype females of taeniarostris revealed that two species - taeniarostris in the sense of Edwards and undescribed species number 10 - were represented. A specimen marked "Type" by Theobald was selected as the lectotype. This female is a typical specimen of the species presently called taeniarostris, so current usage of the name is preserved. Examination of the only extant syntype male of chrysothorax confirmed the currently accepted synonymy of this nominal species with taeniarostris. Study of the types of albicosta, bequaerti, circumluteolus, crassiforceps, luteolateralis, palpalis, and pogonurus confirmed the current usage of these names.

The 10 primary types studied during the fourth contract year bring the total number of holotypes and lectotypes examined to date to 21. The types of seven nominal species remain to be studied.

As the Principal Investigator has become more familiar with the species of Neomelaniconion through the study of newly collected specimens and types, some changes in provisional identifications of specimens borrowed from the British Museum (Natural History) and United States National Museum and reported in the First Annual Report for the project have been made. These changes are: specimens reported as albothorax (Theobald) from Kenya, Sudan, Tanzania, Uganda, and Zaire are actually undescribed species number 9; female specimens reported as crassiforceps from Zaire are actually jamoti Hamon & Rickenbach; specimens

reported as _fuscinervis_ (Edwards) from Gambia and Liberia are undescribed species number 6; specimens reported as _jamoti_ from Liberia are undescribed species number 6; female specimens reported as _palpalis_ from Nigeria are _jamoti_; and female specimens reported as _pogonurus_ from Zaire are _carteri_.

Over the course of this project, provisional keys to the adult females and male genitalia of the species of _Neomelaniconion_ have been developed for the regions of Africa in which the Principal Investigator has done field work. These provisional regional keys have been combined into a pair of provisional keys to the females and male genitalia of all Ethiopian species of the subgenus studied to date (only _ellinorae_ Edwards and _flavimargo_ Edwards are not included). This pair of keys indicates the kinds of morphological characters that the Principal Investigator has found useful in recognizing species of _Neomelaniconion_ and summarizes the taxonomic decisions that have been made. These provisional keys to the females and male genitalia of _Aedes_ (_Neomelaniconion_) in the Ethiopian Region are included in this report as Appendix 1.

The geographic distributions of all Ethiopian species of _Neomelaniconion_ studied are summarized in Table 1. This table is based on all specimens examined to date. It does not include distributions reported in the literature.

During the contract year the phylogenetic (cladistic) relationships of most species of _Neomelaniconion_ were explored using PAUP, which is a computer program for phylogenetic analysis based on the principle of parsimony (36).

The success of such phylogenetic analyses depends on the choice of an outgroup and on the choice and polarization of characters. The choice of an outgroup is not an easy decision to make in the case of mosquitoes because of the confused taxonomy of the family and the virtual absence of a "phylogenetic tree" for the group. In the analysis performed to date, _Aedes_ (_Aedimorphus_) _vexans_ (Meigen) has been used as an outgroup. This choice was made in part because _Aedimorphus_ is similar to _Neomelaniconion_ (both subgenera belong to Subsection 3b of Section B in the classification scheme of Belkin (19)) and in part for the practical reason that detailed descriptions of all stages of this species and specimens are available. It is quite possible that the choice of a different outgroup would result in a much improved cladogram for _Neomelaniconion_. A study of the numerous other subgenera of _Aedes_ has been started with the object of identifying the best outgroup for phylogenetic analysis of _Neomelaniconion_.

The cladistic analyses performed with PAUP to date have utilized morphological characteristics of the adults only and have considered all characters to be of equal value. The consistency indices of these analyses have been very low, in the range of 0.35 to 0.39. This is an indication that many of the characters used in these analyses have no phylogenetic content, that is, they are of no value in tracing the phylogeny of the species. These characters are either too labile to be useful or there has been much parallel and/or convergent evolution. A careful analysis of each character for its phylogenetic content must be made. Those characters found to have little phylogenetic value should be removed from consideration, while those with significant phylogenetic value should be weighted, that is, considered to be of greater value than other characters. The elimination of some characters and the weighting of others will improve the consistency indices of the analyses.

Two cladograms produced with PAUP are included here for reference. The cladogram in Figure 1 shows the phylogenetic relationships of 27 species of *Neomelaniconion* as determined from 35 equally-weighted morphological characteristics of adults with *Aedes vexans* as the outgroup. This cladogram indicates that the Savanna Group of *Neomelaniconion* (the species *albicosta* to *bolensis* Edwards in the upper part of the figure) is monophyletic but that the Forest Group (all remaining species from *bergerardi* Pajot & Geoffroy to *jamoti*) is paraphyletic. The cladogram in Figure 2 shows the phylogenetic relationships of six species of Savanna Group *Neomelaniconion* as determined from the same 35 equally-weighted morphological characteristics of adults with a Forest Group *Neomelaniconion* species, *fuscinervis*, as the outgroup. This figure is included here primarily for comparison with dendrograms of the same six species of *Neomelaniconion* produced from electrophoretic data (Figures 4, 5). However, an additional reason for including this cladogram is to illustrate how the deletion of some species from consideration and a change in the outgroup can completely alter the results of a cladistic analysis. A comparison of the cladogram in Figure 2 with the relationships of the same six species as shown in Figure 1 reveals that there is not a single point of agreement, even though both cladograms were produced from consideration of the same 35 equally-weighted characters.

During the contract year, the Principal Investigator and Assistant spent two and a half weeks at the Vector Biology Laboratory, University of Notre Dame, Indiana, where electrophoretic data for several species of *Neomelaniconion* were obtained. The methods used were those developed at this laboratory by Leonard Munstermann (37,38). One-hundred

eighteen specimens representing 17 populations of six species of Savanna Group Neomelaniconion were stained for 19 cellular enzymes. The species and populations examined were: circumluteolus from Bangui, Bozo, and Talo, Central African Republic, from Bauna, Ivory Coast, and from Mtubatuba and Ndumu, South Africa; luridus McIntosh from Bethulie, South Africa; luteolateralis from Mtubatuba and Oslo Beach, South Africa; mcintoshi from Kedougou, Senegal, and from Mtubatuba, Olifantsvlei, Onderstepoort, and Oslo Beach, South Africa; undescribed species number seven from Villiers, South Africa; and unidentatus from Nairobi, Kenya, and from Olifantsvlei, South Africa. The enzymes tested were aspartate aminotransferase (Aat-2); aconitate hydratase (Aco-1, Aco-2); adenylate kinase (Ak-1, Ak-2); arginine kinase (Ark); esterase (Est); fumarate hydratase (Fum); glycerol-3-phosphate dehydrogenase (Gpd); glucosephosphate isomerase (Gpi); 3-hydroxyacid dehydrogenase (Had-1, Had-2); hexokinase (Hk-2, Hk-3, Hk-4, Hk-C); isocitrate dehydrogenase (Idh-1, Idh-2); lactate dehydrogenase (Ldh); malate dehydrogenase (Mdh-1, Mdh-2); "malic" enzyme (Me-2); mannose phosphate isomerase (Mpi); octanol dehydrogenase (Odh); phosphogluconate dehydrogenase (Pgd); phosphoglucomutase (Pgm); and trehalase (Tre).

Upon return to the University of San Francisco, the raw data obtained at Notre Dame were analyzed over the next several weeks. The number of alleles for each of 20 enzyme loci (Aat-2; Aco-1, -2; Ak-1, -2; Ark; Fum; Gpd; Gpi; Had-2; Hk-C; Idh-1, -2; Ldh; Mdh-1, -2; Me-2; Odh; Pgd; Pgm) was determined and a data matrix of genotype information for 112 individuals (no more than two individuals per family) was prepared and entered into an IBM PC-XT. The genotypic information in this matrix was analyzed with two computer programs, BIOSYS-1 (39) and FREQPARS (40,41).

BIOSYS-1 was used to produce a cluster diagram of the genetic distances among the 17 populations using the unweighted pair-group method based on Nei's (42) unbiased genetic distance coefficient. This cluster diagram is reproduced here as Figure 3. A simplified version of this cluster diagram showing only the genetic distances between the six species analyzed is presented in Figure 4 for comparison with Figures 2 and 5. FREQPARS was used to produce a cladogram of the six species analyzed from allele frequency data. This cladogram is reproduced as Figure 5. Although the cluster diagram of Figure 4 and the cladogram of Figure 5 were constructed from the same data matrix of genotypes, there is little resemblance between them because of the different assumptions and philosophies inherent in the analysis of these data by the two different programs. Neither of these arrangements of the species produced from electrophoretic data resembles the cladogram produced from

adult morphological characters (Figure 2). The absence of congruence among these different estimates of the relationships among the six species of _Neomelaniconion_ analyzed leaves the Principal Investigator questioning whether any of these methods of determining relationships is more reliable than the "intuitive" method of the classical taxonomist.

The primary use of electrophoretic data in mosquito systematics is species discrimination, and many morphologically cryptic or sibling species have been discovered by its use (43-45). Even if electrophoretic data prove to be totally unreliable in estimating the relationships of the species of _Neomelaniconion_, such data could still be of immense value in the detection of morphologically cryptic species and as a check on the Principal Investigator's morphological species concept. The electrophoretic data obtained to date has been useful in both of these latter respects. The five named, morphologically distinct species (_circumluteolus_, _luridus_, _luteolateralis_, _mcintoshi_, _unidentatus_) recognized by earlier workers and by the Principal Investigator have genetic distances of 0.24 to 0.39 from each other (Figure 3), values within the range usually associated with closely related species of _Aedes_ mosquitoes (46-48). With one exception, the populations assigned to each of these species on morphological grounds clustered together at genetic distances of less than 0.01 to 0.06 (Figure 3), values well within the range of inter-populational genetic distances found in other mosquitoes (46-48). The single population with an unusually large genetic distance (0.12) is the Ndumu population of _circumluteolus_. This high genetic distance is undoubtedly an artifact caused by the small sample size (2 individuals) and the fact that several enzymes of these individuals did not stain and so could not be included in the analysis, rather than evidence that this population represents a distinct, morphologically cryptic species.

Even with the acquisition of electrophoretic data, the status of undescribed species number seven is still not resolved. This population is very similar to _mcintoshi_ in all stages, but differs from that species in some colorational features of adults, egg shape, and very subtle male genitalia characters. It was hoped that the electrophoretic data would provide an answer to the status of this population, but these data are equivocal also. This population has no diagnostic alleles for the enzymes tested, but the frequencies of the alleles it has are different enough from other populations of _mcintoshi_ that it joins the cluster of unquestionable _mcintoshi_ populations at a genetic distance of nearly 0.05. While valid biological species, as, for example, _Aedes_ (_Protomacleaya_) _brelandi_ Zavortink, can

have a genetic distance this low (46), such a value is usually associated with only populational differentiation within a single species. If live material of this population and mcintoshi could be reacquired from South Africa, then hybridization experiments between them might provide the definitive answer to the question of the status of the population.

The Principal Investigator would like to utilize electrophoretic data to the fullest extent possible in his biosystematic study of Neomelaniconion, but it will not be possible in to do so under the present contract because of the lack of equipment and adequate time. The electrophoretic work done during this contract year is only about 15% of what could be done with the material that is already frozen and awaiting study. Totally unstudied is frozen material representing three additional Savanna Group species; four additional populations of those Savanna Group species studied this year; and 30 populations of 13 Forest Group species. There are also additional unrelated individuals for many of the populations already studied that could be added to the analysis. Moreover, several enzymes tested at the Vector Biology Laboratory, (Est, Had-1, Mpi, Tre) this year were not included in the data studied to date because there was inadequate time to analyze the gels and score their multiple alleles; additional time is needed to study photographs of the gels produced at the Vector Biology Laboratory in order to be able to utilize the genetic information of these enzymes in the final analysis of variation.

The discoveries that species of the Savanna Group of Neomelaniconion can be maintained in the laboratory by force-mating techniques and that fertile F_1 hybrids between the species can be obtained open up entirely new ways of exploring the genetic relationships of these species. Studies of this type have been done on very few kinds of Aedes mosquitoes (only some species groups of subgenera Stegomyia, Ochlerotatus, and Protomacleaya) (49). It is not possible to pursue this kind of research on Neomelaniconion under the present contract, however, because of its very time-consuming nature. The hybrids produced this year are of value to the present research, though, in that they give some idea of the genetic basis for some of the morphological characters that distinguish the species. Also, some crosses produced few offspring and/or male offspring with abnormal genitalia, and such data can be used to infer the evolutionary relationships of the parental species.

Conclusions

The major conclusions of the comparative morphological taxonomic study of Ethiopian Neomelaniconion are summarized in the provisional keys found in Appendix 1 and the distributional chart shown in Table 1. The keys indicate the species that the Principal Investigator considers to be valid, provide the correct name for each of these as determined by the study of types, indicate the diagnostic features of each species, and show the species composition and defining characteristics of the two major species groups of the subgenus. The distributional chart summarizes the distribution of each species by country, as determined by the examination of all available specimens - types, previously existing museum specimens, and specimens collected specifically for the project.

Other conclusions reached during the contract year are:

1. Many of the characteristics of adult Neomelaniconion that are amenable to cladistic analysis are of little value in indicating the phylogeny of the species.

2. Genetic information obtained through electrophoresis of soluble cellular enzymes is a valuable source of information about the degree of genetic differentiation of populations and species of Neomelaniconion.

3. The cluster diagram and cladogram generated from allele frequency data of Savanna Group species of Neomelaniconion are incongruent with each other and with cladograms generated from morphological characteristics of adults.

4. Force-mating techniques can be used to maintain several Savanna Group species of Neomelaniconion in the laboratory and these same techniques can also be used to produce fertile F_1 hybrids between the species, backcrosses, and F_2 hybrids.

5. The genetic relationships of Savanna Group species of Neomelaniconion could be explored through hybridization experiments.

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Appendix 1.

Provisional Keys to Groups and Species of
Aedes (Neomelaniconion) in the Ethiopian Region

FEMALES

(pogonurus and n. sp. #4 unknown; ellinorae and flavimargo not included)

1. Some or all of terga II-VI with basal light bands (Savanna Group)..2
Terga II-VI without basal light bands (Forest Group)...11

2(1). Acrostichal bristles absent..............................3
Acrostichal bristles present..............................5

3(2). Costa partly white-scaled in distal 0.4; veins R_S and M with light plume scales........................albicosta
Costa completely dark-scaled; veins R_S and M with dark plume scales.......................................4

4(3). Sterna II-VI entirely or predominantly white-scaled; anterior surface of femur III white-scaled in basal 0.5....................................circumluteolus
Sterna II-VI predominantly dark-scaled; anterior surface of femur III white-scaled in basal 0.3........n. sp. #9

5(2). Claws III simple..6
Claws III toothed...8

6(5). Scutum predominantly yellow-scaled; veins R_1, R_{4+5}, and 1A light-scaled..............................aurovenatus
Scutum predominantly dark-scaled in center, with broad, golden-scaled lateral band; veins R_1, R_{4+5} and 1A dark-scaled.......................................7

7(6). Sterna II-VI dark-scaled; anterior surface of femur III inconspicuously light-scaled at base, the scales appearing dark at some angles of observation........... ....mcintoshi (= pallida)
Sterna II-VI white-scaled; anterior surface of femur III conspicuously white-scaled in basal 0.5........n.sp. #7

8(5). Vein R light-scaled almost to apex of R_1...luteolateralis
Vein R light-scaled only to near base of R_S or near level of base of R_{4+5}......................................9

9(8). Lateral band of scutum cream-colored to pale yellow; vein R light-scaled to near base of R_s..............*luridus*
Lateral band of scutum golden-scaled; vein R partly light-scaled to near level of base of R_{4+5}...........10

10(9). Pleural scale patches large; anterior surface of femur III conspicuously white-scaled in basal 0.5...*unidentatus*
Pleural scale patches small; anterior surface of femur III inconspicuously light-scaled at base, the scales appearing dark at some angles of observation...*bolensis*

11(1). Costa with subcostal and apical light-scaled spots.....12
Costa without light-scaled spots........................14

12(11). Scutum predominantly yellow-scaled; posterior surface of femur II and anterior and posterior surfaces of femur III primarily dark-scaled or at least the scales appearing dark at some angles of observation..*n. sp. #1*
Scutum predominantly dark-scaled in center; posterior surface of femur II and anterior and posterior surfaces of femur III conspicuously light-scaled..............13

13(12). Costa extensively yellow-scaled in basal 0.5; *psp* with patch of scales...........................*punctocostalis*
Costa entirely dark-scaled in basal 0.5; *psp* without scales or with only 1 scale.................*maculicosta*

14(11). Wing veins entirely dark-scaled; scutum predominantly dark-scaled, without light-scaled lateral band...*fuscinervis*
Wing vein R and sometimes vein Cu light-scaled at base; scutum with conspicuous light-scaled lateral band....15

15(14). Claws III simple.....................................16
Claws III toothed....................................21

16(15). Tibia III with yellowish-scaled streak in middle dorsally 17
Tibia III dark-scaled in middle dorsally...............19

17(16). Proboscis shorter than femur I, without median light-scaled band; scutum with scattered yellow scales in center, without distinct yellow-scaled inner dorsocentral line...............................*n. sp. #2*
Proboscis longer than femur I, usually with incomplete to complete median yellowish-scaled band; scutum without scattered yellow scales in center, either predominantly yellow-scaled or with distinct, yellow-scaled inner dorsocentral line....................................18

18(17). Scutum predominantly yellow-scaled............<u>bergerardi</u>
Scutum predominantly dark-scaled in center, but with distinct, yellow-scaled inner dorsocentral line..<u>taeniarostris</u>, <u>n. sp. #3</u>

19(16). Center of scutum almost entirely dark-scaled; lateral prescutellar scales predominantly dark; lateral band of scutum usually white-scaled.......................<u>jamoti</u>
Center of scutum with scattered yellow scales or with distinct, yellow-scaled inner dorsocentral line; lateral prescutellar scales yellow; lateral band of scutum yellow-scaled..................................20

20(19). Proboscis with median pale-scaled spot beneath; scutum with distinct yellow-scaled inner dorsocentral line; remigium entirely white-scaled...........<u>crassiforceps</u>
Proboscis entirely dark-scaled in middle; center of scutum with scattered yellow scales; remigium predominantly dark-scaled.....................<u>n. sp. #6</u>

21(15). Tibia III dark-scaled in middle dorsally; center of scutum entirely dark-scaled; lateral band of scutum narrow, white to cream-scaled.................<u>bequaerti</u>
Tibia III light-scaled in middle dorsally; scutum with yellow scales in indistinct to distinct inner dorsocentral line; lateral band of scutum broad, yellow-scaled...22

22(21). Proboscis with submedian pale-scaled spot beneath or incomplete to complete yellow-scaled band............23
Proboscis entirely dark-scaled in middle...............24

23(22). Claws III similar to claws I, II; all claws stout, strongly pigmented, with long, stout tooth......<u>carteri</u>
Claws III unlike claws I, II; claws I, II slender, moderately pigmented, with moderately long, slender tooth; claws III slender, weakly pigmented, with short, triangular tooth............................<u>n. sp. #10</u>

24(23). Claws III similar to claws I, II; all claws abruptly curved beyond long, slender tooth, the distal portion subparallel with tooth........................<u>n. sp. #5</u>
Claws III unlike claws I, II; claws I, II more evenly curved beyond moderately long, slender tooth, the distal portion diverging from tooth; claws III more evenly curved, with short, triangular tooth....<u>palpalis</u>

MALE GENITALIA

(ellinorae and flavimargo not included)

1. Tips of longest teeth at apex of aedeagus widely separated (Savanna Group)............................2
Tips of longest teeth at apex of aedeagus meeting or nearly meeting on midline (Forest Group).............10

2(1). Spiniform setae on mesal surface of sidepiece relatively few (4-13), spread out, inconspicuous, not thicker than largest setae of basal mesal area of sidepiece....... 3
Spiniform setae on mesal surface of sidepiece not as above, either more numerous, or in dense clump, or conspicuous, thicker than largest setae of basal mesal area of sidepiece....................................4

3(2). Sidepiece very broad at level of basal mesal area, abruptly narrowed distad, its mesal margin strongly concave; apical projection of sidepiece relatively narrow, its width at midlength less than 0.5 its length.................................n. sp. #9
Sidepiece not as broad at level of basal mesal area and not as abruptly narrowed distad, its mesal margin slightly concave to nearly straight; if mesal margin slightly concave, then apical projection of sidepiece broad, its width at midlength more than 0.5 its length; if apical projection of sidepiece relatively narrow, its width at midlength less than 0.5 its length, then mesal margin of sidepiece nearly straight..............
.......................mcintoshi (=pallida), n. sp. #7

4(2). Spiniform setae on mesal surface of sidepiece in dense clump arising from a slightly convex area of sidepiece
....5
Spiniform setae on mesal surface of sidepiece spread out, or, if in a relatively dense clump, then not arising from a convex area of sidepiece......................7

5(4). No long setae of basal mesal area of sidepiece arising basad of level of apex of basal mesal lobe.....bolensis
Some long setae of basal mesal area of sidepiece arising basad of level of apex of basal mesal lobe...........6

6(5). All setae of basal mesal area of sidepiece subequal in stoutness................................luteolateralis
More dorsal of setae of basal mesal area of sidepiece slightly finer than more ventral setae........albicosta

7(4). Apical projection of sidepiece very broad, its width at midlength more than 0.5 its length; spiniform setae on mesal surface of sidepiece relatively inconspicuous, only slightly thicker than largest setae of basal mesal area, and subequal in length to width of apex of apical projection of sidepiece.................... aurovenatus

Apical projection of sidepiece narrow, its width at midlength equal to or less than 0.5 its length; spiniform setae on mesal surface of sidepiece conspicuous, much thicker than largest setae of basal mesal area, and at least some longer than width of apex of apical projection of sidepiece.....................8

8(7). No long setae of basal mesal area of sidepiece arising basad of level of apex of basal mesal lobe... unidentatus

Some long setae of basal mesal area of sidepiece arising basad of level of apex of basal mesal lobe............9

9(8). Clasper with spicules............................. luridus

Clasper without spicules.................. circumluteolus

10(1). Clasper with setae....................................11

Clasper without setae..................................17

11(10). Sidepiece with dense masses of long bristles on ventral and dorsolateral surfaces............................12

Sidepiece without dense masses of long bristles........13

12(11). Clasper much enlarged apically, with dense mass of setae around spiniform; apical projection of sidepiece large, with many setae; basal mesal lobe with only 1 spiniform seta............................... pogonurus

Clasper subparallel-sided to near apex, then abruptly narrowed, with relatively few (6-8) setae in distal 0.5; apical projection of sidepiece very small, with only 2-4 setae; basal mesal lobe with 2 spiniform setae..................................... crassiforceps

13(11). Basal mesal area of sidepiece with relatively few (7-12) setae; apical projection of sidepiece short, with few (4-8) setae................................ n. sp. #5

Basal mesal area of sidepiece with dense mass of setae; apical projection of sidepiece long, with numerous (12-16) setae.....................................14

14(13). Spiniform setae on mesal surface of sidepiece 3,4; no rows of fine setae present dorsad of spiniform setae... 15

Spiniform setae on mesal surface of sidepiece 5-8; 2 or 3 rows of fine setae present dorsad of spinform setae..16

15(14). Lateral edges of the 2 areas of dense setae in basal mesal areas of sidepieces subparallel; setae in basal mesal area of sidepiece very dense, their alveoli touching..carteri

Lateral edges of the 2 areas of dense setae in basal mesal areas of sidepieces divergent distad; setae in basal mesal area of sidepiece less dense, their alveoli separated.....................................n. sp. #10

16(14). Spiniform setae on mesal surface of sidepiece very strongly-developed, becoming thicker beyond their bases, the distance between some of them at their widest parts less than their diameters.........palpalis

Spiniform setae on mesal surface of sidepiece strongly developed, not or only very slightly thicker beyond their bases, the distance between them greater than their diameters..............................bequaerti

17(10). Clasper with many conspicuous spicules.................18

Clasper without spicules...................................21

18(17). Spiniform setae on mesal surface of sidepiece in dense clump; setae of basal mesal area moderately dense...... ....n. sp. #3

Spiniform setae on mesal surface of sidepiece spread out; setae of basal mesal area sparse.....................19

19(18). Some (3,4) spiniform setae on mesal surface of sidepiece conspicuously stronger and longer than others; apicomesal portion of ventral surface of sidepiece without moderately- or strongly-developed setae........ ....n. sp. #2

No spiniform setae on mesal surface of sidepiece conspicuously stronger and longer than others; apicomesal portion of ventral surface of sidepiece with few to numerous moderately- to strongly-developed setae 20

20(19). Apicomesal portion of ventral surface of sidepiece with relatively few moderately-developed setae.....n. sp. #6

Apicomesal portion of ventral surface of sidepiece with numerous moderately- to strongly-developed setae....... ....fuscinervis

21(17). Mesal surface of sidepiece without thickened spiniform setae; clasper very conspicuously broadened in middle and its spiniform very long, slender, curved........... ....taeniarostris

Mesal surface of sidepiece with obvious, thickened spiniform setae; clasper usually not conspicuously broadened in middle, or, if it is, then its spiniform shorter, stout, nearly straight.....................22

22(21). Clasper very conspicuously broadened in middle, abruptly narrowed to slender apex not much broader than its spiniform.......................................n. sp. #1

Clasper not or only slightly broadened in middle; if slightly broadened, then not abruptly narrowed to slender apex that is not much broader than its spiniform...23

23(22). Sidepieces nearly parallel to each other; sidepiece broad to far beyond middle, then abruptly narrowed, its mesal margin strongly concave; spiniform setae on mesal surface of sidepiece few (2-7), widely-spaced, from conspicuously protuberant alveoli....................24

Sidepieces diverging from each other distad; sidepiece gradually narrowing from base to apex, its mesal margin nearly straight to slightly concave beyond middle; spiniform setae on mesal surface of sidepiece more numerous (10-18), closely-spaced, in clump or 2 or 3 rows, their alveoli not conspicuously protuberant....25

24(23). Spiniform setae on mesal surface of sidepiece 2; apical projection of sidepiece very broad, with setae restricted to outer edge of ventral surface; clasper tapering to slender apex not much broader than its spiniform; mesal surface of sidepiece with dense mass of setae where sidepiece narrows.............bergerardi

Spiniform setae on mesal surface of sidepiece 5-7; apical projection of sidepiece narrow, with setae distributed over entire ventral surface; clasper not tapering, its apex broad, irregular, jagged; mesal surface of sidepiece without dense mass of setae...........jamoti

25(23). Setae of IX-T long; apical projection of sidepiece with single moderately strong seta...........punctocostalis

Setae of IX-T short; apical projection of sidepiece with several moderately strong setae.....................26

26(25). Spiniform setae on mesal surface of sidepiece in moderately dense clump; more ventral spiniform setae 2.0X length of dorsal ones; apical projection of sidepiece moderately large; setae in basal mesal area of sidepiece moderately dense..............maculicosta

Spiniform setae on mesal surface of sidepiece more spread out; more ventral spiniform setae 1.0-1.3X length of dorsal ones; apical projection of sidepiece small; setae in basal mesal area of sidepiece sparse...n. sp. #4

	albicosta	aurovenatus	bequaerti	bergerardi	bolensis	carteri	circumluteolus	crassiforceps	fuscinervis	jamoti	luridus	luteolateralis	maculicosta	mcintoshi	palpalis	pogonurus	punctocostalis	taeniarostris	unidentatus	n. sp. #1	n. sp. #2	n. sp. #3	n. sp. #4	n. sp. #5	n. sp. #6	n. sp. #7	n. sp. #9	n. sp. #10
Angola														●														
Basutoland																			●									
Bechuanaland														●														
Benin																												
Burkina Faso					●					●																		
Burundi																												
Cameroon																												
Central African Republic				●	●	●	●	●	●	●					●	●		●		●		●		●				
Congo																												
Equatorial Guinea																												
Ethiopia	●						●							●														
Gabon																												
Gambia							●							●											●			
Ghana					●		●		●								●	●										●
Guinea																												
Guinea Bissau																												
Ivory Coast							●		●	●			●				●	●		●	●	●	●					●
Kenya	●						●							●					●								●	
Liberia																	●								●			
Malawi							●																					
Mozambique							●																				●	
Nigeria						●	●		●	●			●	●			●	●										
Rwanda							●																					
Senegal					●		●			●				●														
Sierra Leone																												
Somalia	●																											
South Africa		●					●				●	●		●					●							●		
South-West Africa																												
Sudan							●							●													●	
Swaziland																												
Tanzania														●													●	
Togo																												
Uganda							●								●												●	
Zaire			●			●	●	●		●				●	●	●											●	
Zambia			●				●							●														
Zimbabwe														●					●									

Table 1. Geographic distributions of species of Aedes (Neomelaniconion) in the Ethiopian Region (ellinorae and flavimargo not included).

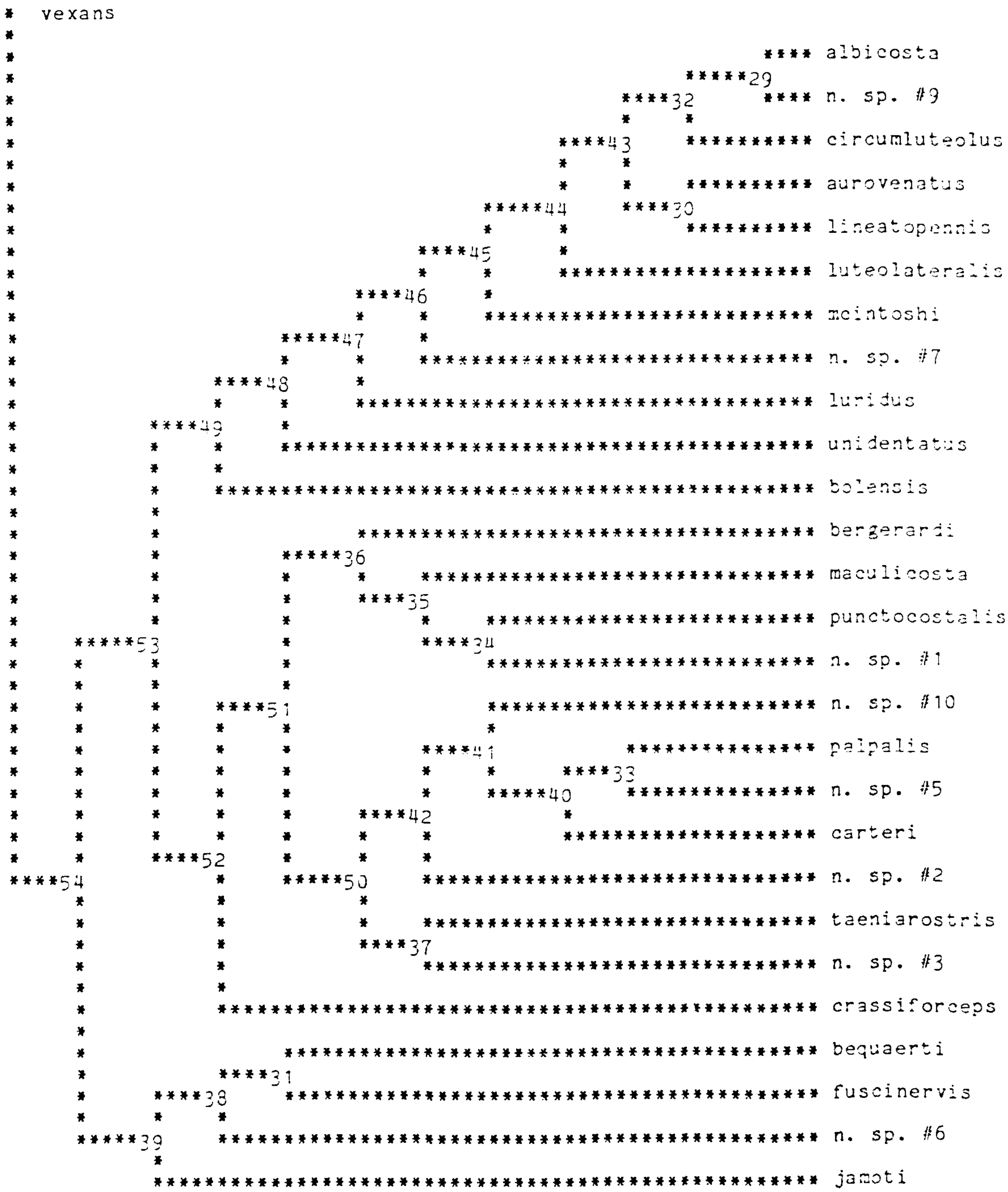

Figure 1. Cladogram of 27 species of <u>Neomelaniconion</u>. Cladogram generated from 35 equally-weighted adult characteristics using the principle of maximum parsimony with <u>Ae</u>. <u>vexans</u> as outgroup.

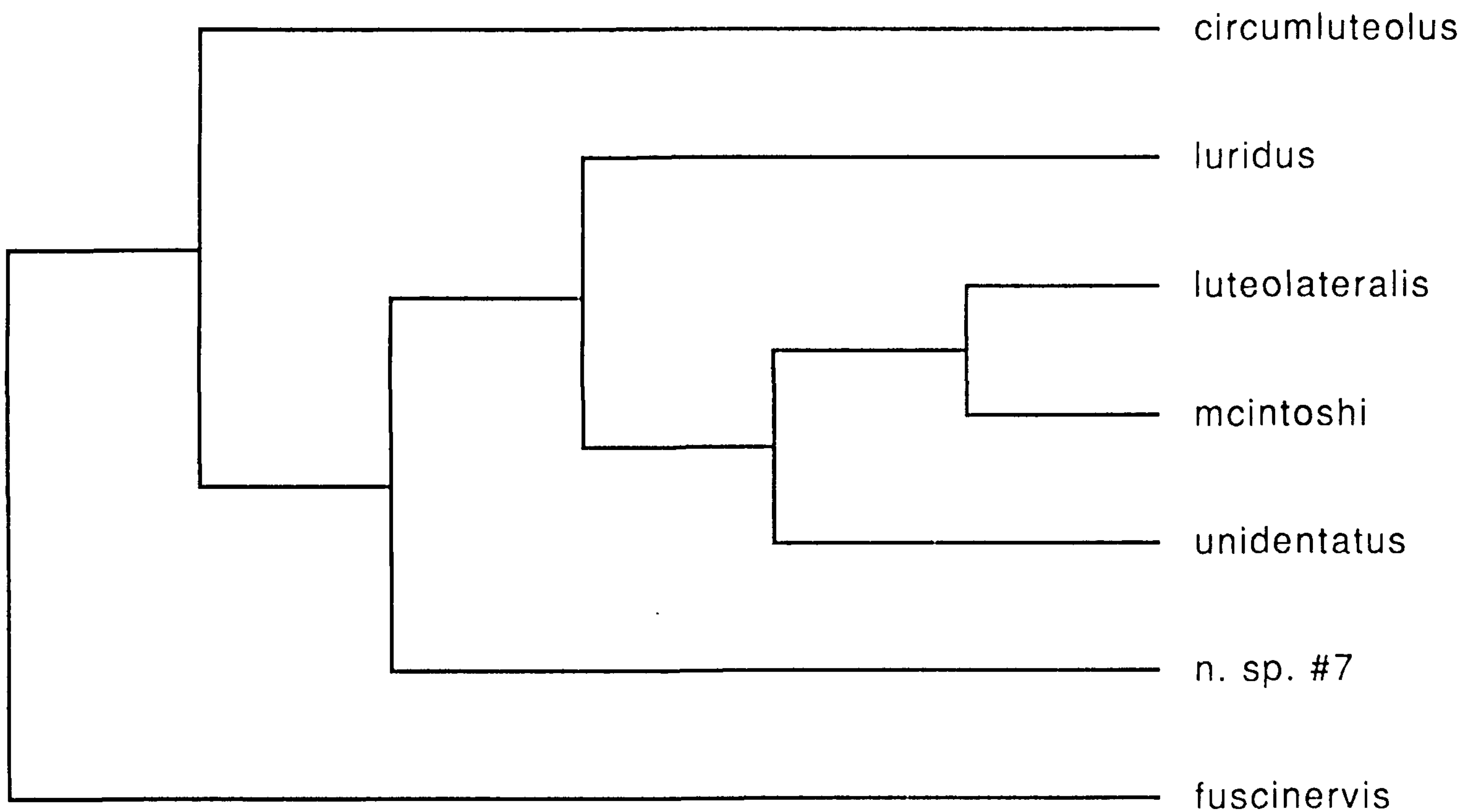

Figure 2. Cladogram of 6 species of Savanna Group Neomelaniconion. Cladogram generated from 35 equally-weighted adult characteristics using the principle of maximum parsimony with Ae. fuscinervis as outgroup.

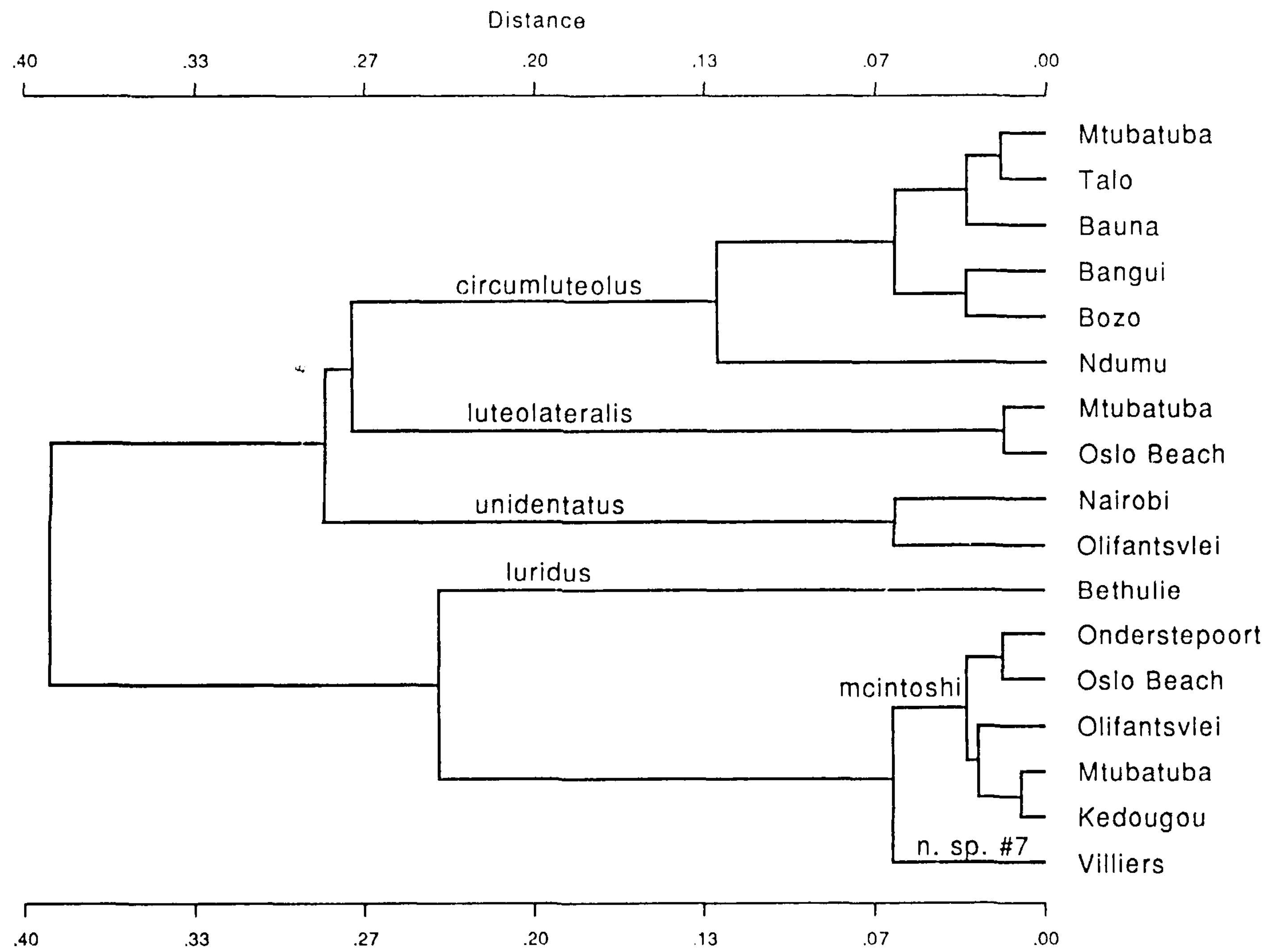

Figure 3. Cluster diagram of 17 populations of Savanna Group Neomelaniconion. Cluster diagram generated from allele frequency data using the unweighted pair-group method of clustering of the unbiased genetic distance coefficient of Nei.

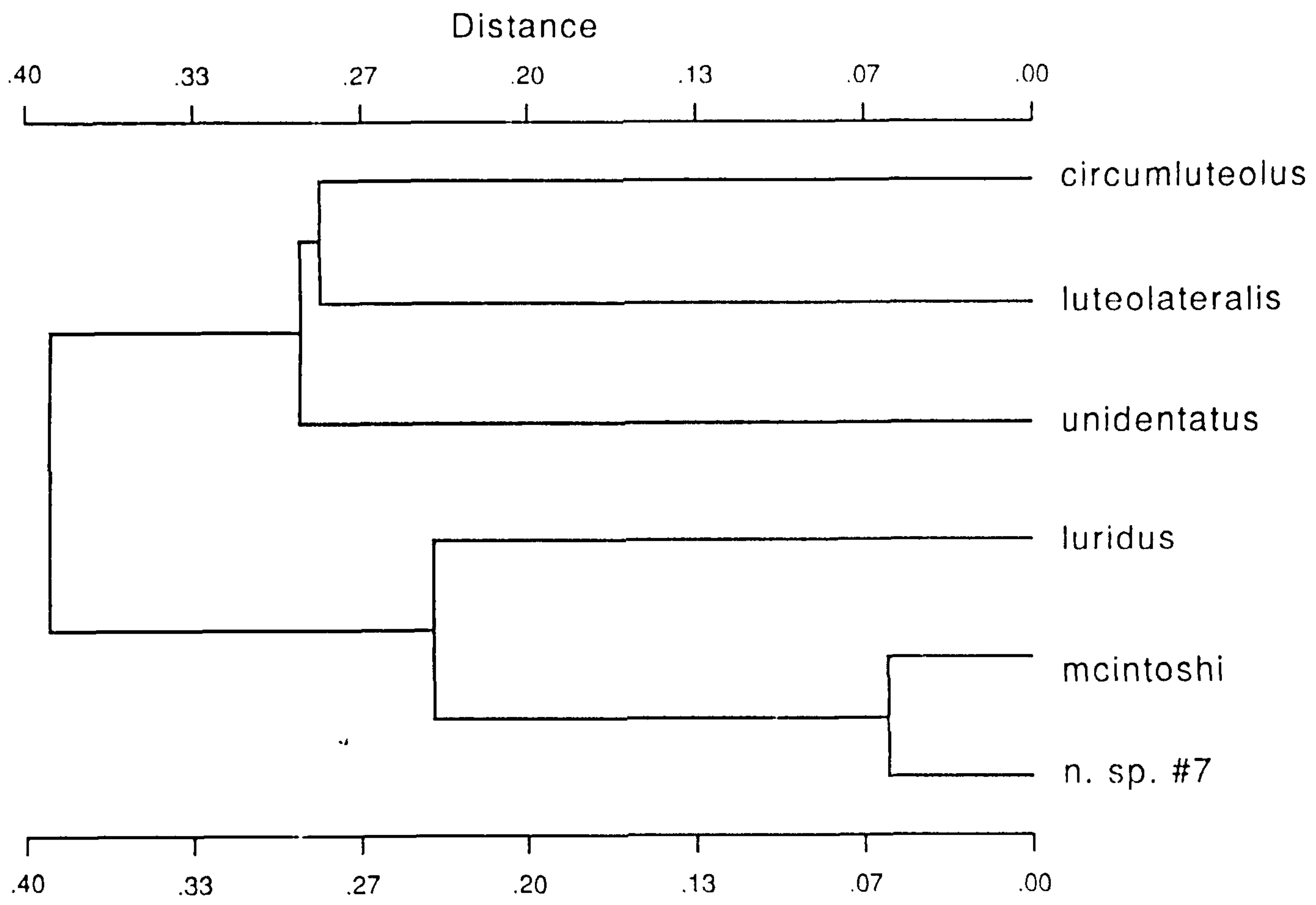

Figure 4. Cluster diagram of 6 species of Savanna Group Neomelaniconion. Cluster diagram generated from allele frequency data using the unweighted pair-group method of clustering of the unbiased genetic distance coefficient of Nei.

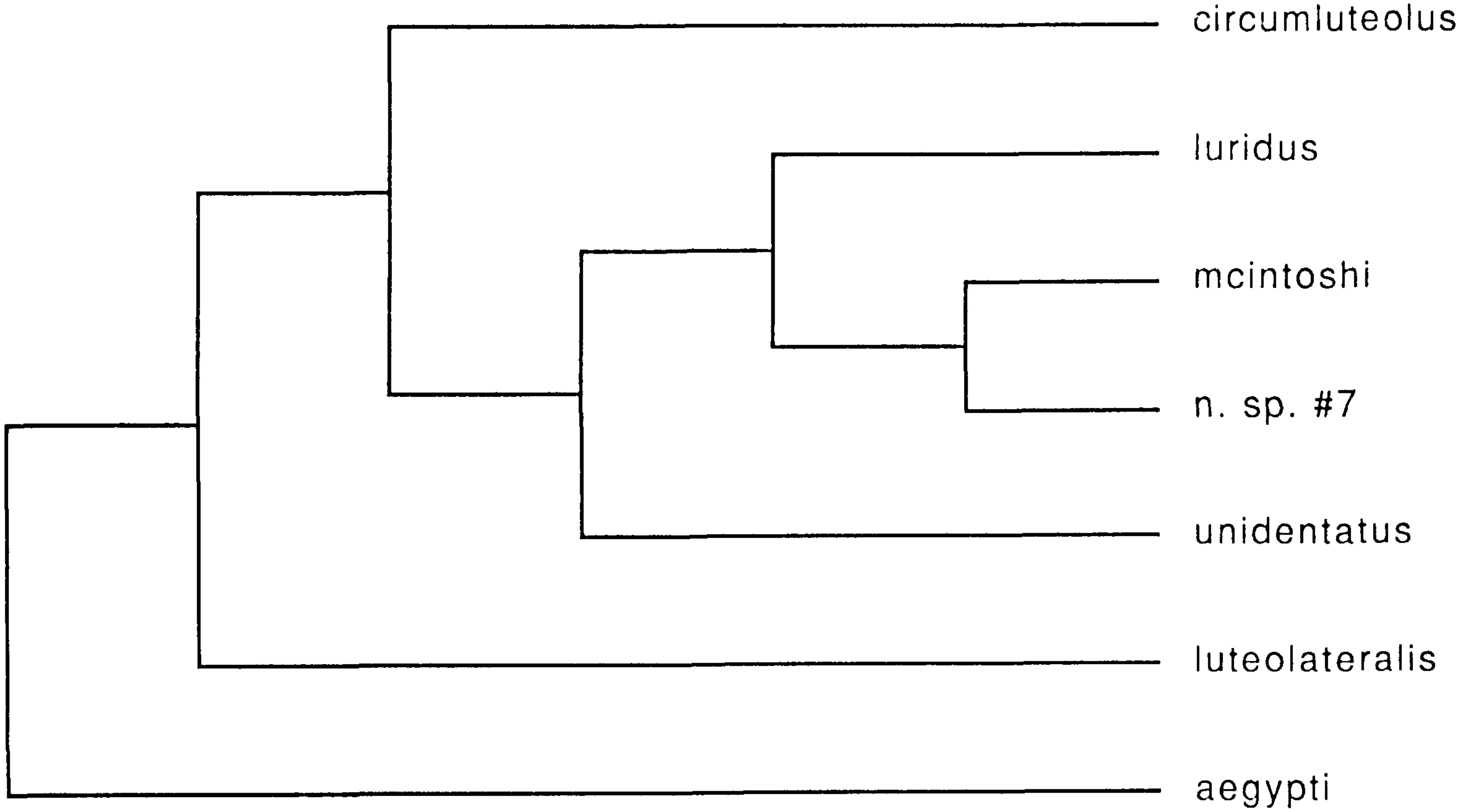

Figure 5. Cladogram of 6 species of Savanna Group Neomelaniconion. Cladogram generated from allele frequency data using principle of maximum parsimony.

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